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13. ABSTRACT (Maximum 200 words)

The primary objectives of the project on Research on Antimalarial Chemotherapy: "Novel approach to combination therapy in multidrug resistant malaria" focused on confirmation of reversal of chloroquine resistance in isolates of *P. falciparum* from Nigeria and molecular biology, pharmacokinetics and biochemistry of the reversal phenomenon. The major objectives of the project were accomplished. Efforts during the project resulted in modification of standard *in vitro* techniques for continuous cultivation of *P. falciparum* in Nigeria. The modified technique is now in routine use at the cooperative malaria laboratory in the department of Pharmacology and Therapeutics University of Ibadan, Nigeria. Reversal of mefloquine resistance with penfluridol and reversal of chloroquine resistance with promethazine (WRBL 50601), chlorpheniramine, diphenhydramine and pyrilamine were identified and published in 1991 and 1992. The findings on reversal of chloroquine resistance with promethazine and chlorpheniramine represent the first demonstration of the reversal phenomenon with drugs prescribed as adjunct in chemotherapy of malaria in an endemic area. Subsequent efforts were allocated to studies on pharmacokinetics and clinical efficacy of potential combinations of chlorpheniramine with antimalarial drugs in human volunteers and animal models. Other studies accomplished during the period include clinical efficacy of artemether, mefloquine and suphadoxine-pyrimethamine. Nine manuscripts have been published, two are being prepared for publication.

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Angela Odoba 02/14/96

PI - Signature Date

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I Introduction and Summary of Achievement of the Objectives of the Grant

Ia. The objectives of the project are:

1. To evaluate the clinical efficacy of combination therapy with chloroquine and reversing compounds (chlorpheniramine or promethazine) in treatment of chloroquine resistant malaria.

2. To evaluate and confirm reversal of resistance to chloroquine, quinine, quinidine and mefloquine with verapamil or penfluridol in isolate and clones of *P. falciparum* obtained from Nigeria. Correlate reversal of CQR with clinical response of the patients and use the unique reversal with verapamil in enhancing a technique for detection of CQR *P. falciparum*.

3. To evaluate effects of co-administration of reversing compounds on pharmacokinetic properties of standard antimalarial drug.

Ib. Achievement of the Grant

This represents the final report on project (Cooperative Agreement DAMD17-93-V-3002) WHO/TDR 1D # 890178 (Oct. 1992 to Dec. 1994). In order to avoid repetition of work already enumerated in publications and earlier reports, a brief summary of the achievements of the project is provided with emphasis on achievements not previously reported. The primary objectives of the project on Reseach on Antimalarial Chemotherapy :"Novel approach to combination therapy in multidrug resistant malaria" remained unchanged. It focused on confirmation of reversal of chloroquine resistance in isolates of *P. falciparum* from Nigeria and studies on molecular biology, pharmacokinetics and biochemistry of the reversal phenomenon. The major objectives of the project were accomplished during the grant period.

Efforts during the project resulted in modification of standard *in vitro* techniques for continuous cultivation of *P. falciparum* in Nigeria. The modified technique is now in routine use at the cooperative malaria laboratory in the department of Pharmacology and Therapeutics University of Ibadan, Nigeria. Reversal of mefloquine resistance with penfluridol and reversal of chloroquine resistance with promathazine (WRBL 50601), chlorpheniramine, diphenhydramine and pyrilamine were identified and constitute part of reports published in 1991 and 1992. Subsequently, reversal of chloroquine resistance with promethazine and chlorphenirame have been confirmed in additional isolates of *P. falciparum* obtained from patients in Nigeria. This finding represents the first demonstration of the reversal phenomenon with drugs prescribed as adjunct in chemotherapy of malaria in an endemic area. The significance of these observations influenced allocation of subsequent efforts to studies on pharmacokinetics and clinical efficacy of potential combinations of chlorpheniramine with antimalarial drugs in human volunteers and animal models. Chlorpheniramine was selected for further studies because of the milder side effects compared with promethazine. Major activities not previously reported or published are outlined below.

1. Confirmation of reversal of chloroquine resistance and identification of reversal of quinine resistance with chlorpheniramine and promethazine in isolates of *P. falciparum* adapted to continuous culture in Nigeria.
2. Studies on Pharmacokinetics interactions of chlorpheniramine and acetaminophen with quinine.
3. Studies on Pharmacokinetics interaction of promethazine with chloroquine.
4. Studies on the clinical efficacy of a combination of chloroquine with chlorpheniramine in the treatment of CQR malaria.

In addition to these studies on the clinical application of the reversal phenomenon, efforts were devoted to studies on.

1. Clinical studies on efficacy of artemether, mefloquine and suphadoxine-pyrimethanine.
2. Studies on parasite ring viability following antimalarial drug administration.

A total of nine manuscripts have been published while additional two are being prepared for publication. A total of eleven students (7 Ph.D and 5 M.Sc) were trained during the period of the grant. In addition 8 technical staff received training in various techniques for studies on malaria. One WHO post doctoral fellow (Dr. I. Obarisiagbon) started training on techniques for continuous cultivation of *P. falciparum* and isoenzyme analysis for studies on molecular biology of chloroquine resistant *P. falciparum*.

II Research Activities

STUDIES ON THE PHARMACOKINETICS OF QUININE COMBINATIONS.

The Pharmacokinetic disposition of quinine and combinations with either tetracycline or paracetamol were studied after oral administration to healthy African subjects. All study protocols were approved by the Joint University College Hospital and College of Medicine University of Ibadan ethical review committee. Twenty-one healthy male adults aged between 21 and 39 years volunteered and participated in the study after giving written informed consents. The volunteers were non-smokers who consumed alcohol only occasionally. They weighed between 58 and 74kg. Each subject was selected for participation based on normal findings on physical examination and normal urinalysis, biochemical and hematological tests. In addition, they did not take any medication for 4 weeks prior to participation and during the period of the study.

The subjects fasted overnight before the study up till 3 hours after drug administration. A fore-arm vein was cannulated for blood sampling and kept patient with heparinized normal saline (heparin 5iu/ml). The Drug(s) were administered at 3 week intervals by a randomized cross-over design.

Drug regimen:

(i) Quinine/Paracetamol

- Six subjects received either oral quinine sulphate (600mg) or oral paracetamol (1000mg) 6 hourly for the first 3 doses followed by a combination of paracetamol (1000mg) and quinine sulphate (600mg) after a 12 hour (overnight) fast.
- This regimen was designed to simulate as closely as possible the usual self treatment history of the typical patient before seeking medical treatment.

- (ii) Quinine/Chloropheniramine

- Eight subjects received either oral quinine sulphate (600mg) or oral chlorpheniramine (4mg) followed by a combination of quinine sulphate (600mg) and chlorpheniramine (4mg).

- (iii) Quinine/Tetracycline

- Seven subjects received either oral quinine sulphate (600mg) or tetracycline (250mg) followed by a combination of quinine sulphate (600mg) and tetracycline (250mg).

- Sample collection.

- Venous blood was obtained before and at 0.25, 0.5 0.75, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0 36.0 and 48.0 hours after drug administration.
- The plasma and red cells were separated after centrifuging at 1200 rpm for 10 minutes and stored at -20°C. Each subjects blood pressure and pulse rate were monitored every hour for the first 12 hours after drug administration and subsequently at the time of blood sampling. The subjects were questioned for any subjective feeling during the first 12 hours after drug administration.

IIb. Analysis:

- The concentration of quinine in plasma was measured by a high-performance liquid chromatography method using a reverse phase column and a fluorescence detector (Sowunmi and Salako 1992).
- The intra and inter assay co-efficients of variation were 5.5 and 2.5% for the concentrations of 10ng/ml and 10ug/ml respectively. Recoveries over the concentration range of 10ng/ml to 10ug/ml were 93-10%. The lower limit of detection was 20ng/ml. Callibration plots were linear ($r = 0.9897$) up to 8 ug/ml.
- The pharmacokinetic parameters calculated from the concentration time data are presented in tables. The pharmacokinetic parameters of quinine (500mg base) following a single intramuscular injection (7 volunteers)or oral (11 volunteers)administration in healthy Nigerian volunteers are shown on Table 1.

- There were no significant differences ($p > 0.05$) in the maximum plasma concentration when quinine was administered alone or incombination with paracetamol (Cmax = 2.10 $\pm$ 0.5 ug/ml vs 2.50 $\pm$ 0.8 ug/ml; Tmax = 4.3 $\pm$ 2.8h vs $\pm$ 4.4 $\pm$2.2 hr respectively). The mean elimination half life of quinine when administered alone (10.5$\pm$3.7h) was similar to that obtained when co-administered with paracetamol (9.5+3.7h). The oral clearance of quinine and the area under the plasma concentration time cure was also not significantly

different when quinine was administered alone 0.26 + 0.09 L/h/kg and 39.1+15.1 ug/ml.h respectively) or when co-administered with paracetamol (0.23 ± 0.03 l/h/kg and 40.7 ± 6.1 ug/ml.h respectively) (Table 2). The data obtained from the subjects studied (n=6) indicate that co-administration of paracetamol with quinine in therapeutic doses does not significantly affect the disposition kinetics of quinine in healthy young adults.

- The interaction between quinine and the resistance reversing compound chlorpheniramine was studied in 8 subjects. Plasma quinine Cmax was increased from 2.9+0.7 mg/l when quinine was administered alone to 3.1 + 1.1 mg/l after co-administration of chlorpheniramine. Similarly the elimination half life increased from 12.6 ± 5.5h. to 13.5 ± 4.6h when chlorpheniramine was co-administered with quinine. The volume of distribution was however reduced from 2.63 ± 0.85 L/kg to 2.39 ± 0.55 L/kg following administration of quinine / chlorpheniramine combination. These differences were not statisfically significant. There were however significant reductions in quinine AUC_{0-t} and plasma clearance (Clp) by the combination. (Table 3).

- Combination of quinine/chlorpheniramine significantly increased quinine profile in the red cell. There were significant increases in Cmax, T1/2 Tmax and AUC values following co-administration of chlorpheniramine (Table 4). The combination was however well tolerated in all subjects studied.
- Co-administration of tetracycline with quinine did not produce any significant changes in quinine pharmacokinetic profile. (Table 5).

- Data obtained from studies on chloroquine/promethazine interaction are currently being analysed.

IIc. STUDIES ON THE EFFECT OF CHLORPHENIRAMINE ON *IN-VITRO/IN-VIVO* SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* TO CHLOROQUINE IN SOUTH WEST NIGERIA.

IN VIVO STUDY.

- Patients (under 5 years of age) with clinical symptoms and microscopically confirmed *P. falciparum* infection were randomly allocated into one of two treatment groups if following criteria were fulfilled.

(1) Pure asexual *P. falciparum* infection > 8,000 parasites per ul blood.

(2) Negative history of antimalarial drug ingestion in preceding 3 weeks.

(3) Negative dill glazko and lignin tests.

(4) Parent/guardian consent.

- One group received chloroquine alone (25mg/kg body weight) given in 3 doses, while the other group received a combination of chloroquine (given as above) and chlorpheniramine (6mg in three divided doses). Parasitological and clinical response of

each patient to treatment were monitored using the WHO extended 28-day test. Thick and thin blood films were prepared for each patient daily for 3 consecutive days and subsequently weekly till day 28. Blood samples were obtained from each patient before treatment and used for WHO standard schizont inhibition assay.

IN-VIVO RESPONSE

Nineteen (19) out of the initial 30 patients enrolled completed the study. Eight (8) patients were treated with chloroquine alone while eleven (11) patients received the combination of chloroquine and chlorpheniramine. Cure rate by day 28 was 38% and 64% in the chloroquine and chloroquine/chlorpheniramine group respectively.

IN-VITRO STUDY

Blood samples were obtained from each patient before treatment. An aliquot of each sample was used for *in vitro* drug susceptibility testing using a modification of the WHO schizont inhibitory assay (Reckmann et al., 1978). Ninety-six well microtitre plates containing serial dilutions of chloroquine were modified to incorporate fixed concentrations of chlorpheniramine ($1X10^{-6}M$) or verapamil ($1X10^{-6}M$). Each assay was monitored for 24hr-36hrs. At the end of each experiment giemsa-stained thick blood films were prepared and examined microscopically.

Sixteen (16) of the schizont inhibition assays were successful. Two (2) of the isolates were sensitive to chloroquine *in vitro* using a cut off concentration of 3.55 ug/l. The minimum inhibitory concentrations for chloroquine against the isolates was 2.10 ng/ml. Thirteen (13) isolates were resistant to chloroquine by this criteria. The minimum inhibitory concentrations for chloroquine against the isolates ranged from 6.20 ng/ml to 166.66 ng/ml. Simultaneous exposure of resistant parasites to a combination of chloroquine and a reversing compound increased susceptibility of 7 of the isolates to chloroquine. In contrast, combination of chloroquine with the reversing compound produced an increase in MIC in 2 sensitive isolates (Table 6). Although the implication of a decrease in the activity of chloroquine against a sensitive isolate in the presence of a reversing compound is not clear, it has been previously observed in cultured isolates (Kyle et al., 1990)

Complete *in vivo* and *in vitro* data were obtained for 10 patients. Six (6) of them received chloroquine alone while 4 patients were treated with the combination of chloroquine and chlorpheniramine (CQ/CP). In the CQ group 3 patients were cured of infection while recrudescense occured in 2 patients and infection in one patient failed to respond to treatment. *In vitro* data for these patients showed that 3 isolates were sensitive and 3 isolates were resistant to chloroquine. Chlorpheniramine did not exhibit any effect on the activity of chloroquine against these resistant parasites *in vitro*. In the CQ/CP group (4), three (3) of the patients were cured of infection while infection in the fourth patient failed to respond to therapy. *In vitro* data for the patients showed that 3 of the isolates of P. falciparum were resistant to chloroquine while only one isolate was sensitive.

Chlorheniramine markedly reduced MIC for chloroquine against the resistant parasites *in vitro*.

The results indicate that chlorpheniramine enhances the intrinsic antimalarial activity of chloroquine against chloroquine resistant parasites. However a larger study group is required to confirm this observation against chloroquine resistant malaria infections. The study is currently being repeated in a larger population using a double blind placebo controlled design. Results of the study would constitute an addendum to this report.

III PUBLICATIONS

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(2) Sowunmi, A., ODUOLA, A.M.j., Salako, L.A., Ogundahunsi, O.A.T. and Laoye, O.J. (1992). The relationship between response of *Plasmodium falciparum* to mefloquine in African children and its sensitivity *in vitro*. Transaction of the Royal Society of Tropical Medicine and Hygiene. 86: 368-371.

(3) ODUOLA, A.M.J., Omitowoju, G.O., Garena, L., Kyle, D.E., Milhus, W.k., Sowunmi, A. and Salako, L.A. (1993). Reversal of mefloquine resistance with penfluridol in isolates of *Plasmodium falciparum* from southwest Nigeria. Transaction of the Royal Society for tropical medicine and Hygiene 87:81-83.

(4) ODUOLA, A.M.J., Ogundahunsi, O.A.T. and Salako, L.A. (1992). Continuous cultivation and drug susceptibility testing of *Plasmodium falciparum* in malaria endemic area. Journal of Protozoology 39(5) 605-608.

(5) A. Sowunmi, L.A. Salako, A.M.J. ODUOLA, O, Walker, J.A. Akindele and O.A.T. Ogundahunsi. (1993). Neuropsychiatric side effects to mefloquine in Africans Transactions of the Royal society for tropical medicine and Hygeine 87:462-463.

(6) A. Sowunmi, J.A. Akindele, G.O. Omitowoju, A. Omigbodum, ODUOLA A.M.J and Salako L.A (1993). Intramuscular sulphadoxine- Pyrimethamine in uncomplicated chloroquine-resistant falciparum malaria during pregnancy. Transactions of the Royal Society for Tropical Medicine and hygiene 87:472.

(7) Sowunmi, A., **ODUOLA**, A.M.J.,Ilesanmi, A.O. and Salako, L.A. 1994. Open comparison of artemether and mefloquine in uncomplicated *Plasmodium falciparum* hyperparasitaemia in children. Annal Trop. Paediatr. Accepted for Publication.

(8) Salako, L.A., Walker, O., Sowunmi, A., Omokhodion S. I., Adio. R.A. and **ODUOLA**, A.M.J. 1994. Artemether in severe and cerebral malaria in Nigerian Children. Trans. Roy. Soc. Trop. Med. Hyg., 88: Supplement 1. 13-15

(9) Sowunmi, A. and **ODUOLA**, A.M.J. 1994. Open comparison of mefloquine, mefloquine-sulfadoxine-pyrimethamine and chloroquine in acute uncomplicated falciparum malaria in children. Trans. Roy. Soc. Trop. Med. Hyg., 89:303.

IV Staff Receiving Remuneration and Development Training

(i) **Mrs. R.A. Adio** (Chief technologist) received training between July-and August-1991 on application of the isolated perfused rat liver system. Her training was at the department of Pharmacology, University of Sheffield and Division of Experimental Therapeutics, WRAIR Washington D.C. USA.

(ii) **Mr. K.T. Babalola** (AIMLT, M.Sc). Completed training in the techniques for preparation of DNA from isolates of *P. falciparum*.

(iii) Three technicians with the national diploma in laboratory technology were trained in the laboratory (i) **Mr. A.O. Sijuade** was trained on the use of HPLC for analysis of antimalarial drugs in body fluids. (ii) **Ms. Oyelakin** was trained on techniques for continuous cultivation of *Plasmodium falciparum* and use of the WHO micro test kit. (iii) **Ms. Olaniran** was trained in techniques for continuous cultivation and drug susceptibility testing of *P. falciparum*.

(iv) **Mr. Wole Oyebamiji,** a laboratory trainee assistant has been trained to assist in pharmacokinetics studies in human volunteers and routine microscopy.

(v) Mr. Aina (BSc) and Mr. Thomas (MSc) were trained on techniques for enzyme determination and DNA extraction respectively. In addition they were both trained on techniques for continuous cultivation and drug susceptibility testing of *P. falciparum*.

(vi) Miss Sanni a trainee laboratory technologist is being trained on the technique for continuous cultivation of *Plasmodium falciparum* and use of the WHO micro test kit.

V. In-House Research Training

A total of 11 students (7 Ph.D and 5 M.Sc) were trained or are being trained.

1 **Mr. B. Umotong (Ph.D)**: Primarily attached to the Department of Immunology received training in the use of phase contrast microscopy for IFA. He is currently in the final stages of defending a thesis on "Immunological studies of Nigerians infected with chloroquine resistant *P. falciparum*".

2. **Mr. Hedo (Ph.D)**: Primarily attached to the Department of Immunology received training in the use of phase contrast microscopy for IFA. He is currently in the final stages of defending a thesis on. "Immunological parameters and severity of sickle cell anemia in Nigerian adults".

3. **Mr. Bola Taiwo** (Ph.D): Received training on technique for evaluating cardiotoxicity of reversing compounds alone and in combination with chloroquine using experimental animal models.

4. **Mr. O.A.T. Ogundahunsi** (Ph.D) Received additional training in techniques for (a) *in vitro* continuous cultivation and drug susceptibility testing of P. falciparum (b) screening potential resistance reversing compounds (c) biological assay for evaluating reversing ability of drugs after oral absorption and potential biotransformation (d) Use and modification of the WHO micro test Kit for rapid diagnosis of CQ resistant malaria.

5. **Mr. V. Nwagbarocha** (M.Sc): Completed studies culminating in the award of M.Sc (Pharmacology & Therapeutics). His thesis supported by the lab study project is titled "*In vitro* cultivation of *P. falciparum* using plasma free culture medium".

6. **Dr. C.O Kawesha** (M.Sc) A WHO/TDR trainee received training on conduct of clinical efficacy studies and Pharmacokinetics. His thesis on "Pharmacokinetic interactions of quinine and tetracycline" was successfully defended for the degree of M.Sc in Clinical Pharmacology.

7. **Mr. E Achidi** (Ph.D) Primarily attached to the Department of immunology received training in the use of phase microscopy for IFA.

8. **Miss. G.O. Omitowoju** (Ph.D) Obtained a M.Sc (Pharmacology & Therapeutics) in 1991 based on studies conducted in the lab. She is currently registered for a doctorate with the group.

9. **Mrs. Charles-Davies** (Ph.D) primarly attached to the Department of Chemical pathology. She received training on the use of phase contrast microscopy.

10. **Mr. Wale Oduola** is currently registered for a M.Sc degree in Pharmacology.

11. **Mr. Aina** is registered for an M.Sc degree in Pharmacology based on studies to be conducted in the Laboratory.

TABLE 1

PHARMACOKINETIC PARAMETERS OF QUININE (500 MG BASE) AFTER A SINGLE INTRAMUSCULAR INJECTION OR ORAL ADMINISTRATION IN HEALTHY NIGERIAN ADULTS.

Parameter	Intramuscular Quinine (n = 7)	Oral Quinine (n = 11)
C_{max} (mg/l)	4.50 + 1.30	2.90 + 0.50
t_{max} (hr)	1.70 + 1.10	3.30 + 0.80
$T_{1/2}$ (hr)	10.70 + 3.50	11.70 + 2.90
V_Z (l/kg)	2.18 + 0.99	2.50 + 0.70
Cl_p (l/hr/kg)	0.15 + 0.09	0.15 + 0.04
$AUC_{0-\infty}$(mg hr/l)	71.80 + 29.40	54.90 + 19.

TABLE 2a PLASMA PHARMACOKINETIC PARAMETERS FOR QUININE IN 6 HEALTHY SUBJECTS FOLLOWING ORAL ADMINISTRATION OF 600 MG QUININE SULPHATE.

Volunteer #	Cmax (ug/ml)	Tmax (h)	$t_{1/2}$ (h)	Vd (L/kg)	Clp (Oral) (L/h/kg)	AUC (ug/ml.h)
1	3.1	4	12.6	2.1	0.17	57.6
2	1.9	3	11.9	5.5	0.28	32.9
3	1.9	3	14.9	4.2	0.16	58.5
4	2.0	10	10.9	1.3	0.31	27.9
5	2.3	3	6.8	2.3	0.28	34.3
6	1.6	3	5.4	2.7	0.40	23.7
Mean	2.1	4.3	10.5	3.0	0.26	39.1
s.d.	0.5	2.8	3.7	1.5	0.09	15.1

TABLE 2b PLASMA PHARMACOKINETIC PARAMETERS FOR QUININE IN 6 HEALTHY SUBJECTS FOLLOWING ORAL ADMINISTRATION OF 600 MG QUININE SULPHATE AND 1000 MG PARACETAMOL.

Volunteer #	Cmax (ug/ml)	Tmax (h)	$t_{1/2}$ (h)	Vd (L/kg)	Clp (Oral) (L/h/kg)	AUC (ug/ml.h)
1	3.5	1.5	8.9	2.2	0.20	48.4
2	1.5	6	8.8	2.4	0.25	37.0
3	3.4	4	12.1	1.8	0.24	39.2
4	1.8	8	13.7	2.1	0.18	48.0
5	2.5	3	7.0	2.1	0.25	38.3
6	2.3	4	6.9	1.8	0.28	33.5
Mean	2.5	4.4	9.5	2.1	0.23	40.7
s.d.	0.8	2.2	2.7	0.2	0.03	6.1

TABLE 3a. PLASMA PHARMACOKINETIC PARAMETERS AFTER A SINGLE ORAL DOSE OF 500MG QUININE BASE WITH 4MG CHLORPHENIRAMINE.

Subject	Weight kg	Cmax mg L-1	tmax h	$t1/2_2$ h	Vz Lkg^{-1}	CLP $Lhkg^{-1}$	AUc_0 mgh L-1	$AUC^{0}00$ Mg h L-1
1	67	2.9	4	18.0	2.38	0.09	6.68	79.8
2	62	3.1	3	9.2	2.39	0.18	42.8	79.8
3	60	2.2	4	15.8	3.10	0.13	53.1	59.9
4.	74	1.1	6	9.4	3.31	0.24	25.8	27.1
5	61	4.4	4	19.8	1.71	0.06	111.2	134.1
6	64	3.0	1	8.4	2.25	0.18	38.8	41.2
7	58	4.4	6	10.4	1.87	0.11	72.6	75.6
8	62	3.4	3	17.3	2.16	0.08	81.5	69.1
mean	63.5	3.1	3.8	13.5	2.39	0.13	61.6	69.1
SD	5.0	1.1	1.6	4.6	0.55	0.06	27.3	34.0

TABLE 3b. PLASMA PHARMACOKINETIC PARAMETERS AFTER A SINGLE ORAL DOSE OF 5000MG QUININE BASE ALONE.

Subject	Weight kg	Cmax mg L-1	tmax h	$t_{1/2}$ h	Vz Lkg^{-1}	CLP $Lhkg^{-1}$	AUc_0 mgh L-1	$AUC^{0\infty}$ Mg h L-1
1	67	2.8	2	7.8	2.2	0.18	49.1	40.7
2	62	3.0	2	10.2	2.39	0.16	47.4	48.9
3	60	1.7	4	16.8	4.36	0.18	40.8	45.7
4	74	3.7	4	8.3	2.49	0.20	31.2	31.9
5	61	4.2	4	24.1	1.78	0.05	122.2	156.9
6	64	2.3	4	8.4	3.38	0.28	26.9	27.8
7	58	2.9	4	12.5	2.62	0.14	54.9	58.5
8	62	3.3	4	13.1	2.00	0.10	69.1	74.8
mean	63.5	2.9	3.5	12.6	2.63	0.16	54.0	60.6
SD	5.0	0.7	0.9	5.5	0.85	0.06	30.5	41.6

TABLE 4. RED BLOOD CELL PHARMACOKINETIC PARAMETERS AFTER A SINGLE ORAL DOSE OF 500MG QUININE BASE WITH 4MG CHLORPHENIRAMINE.

Subject	Weight kg	Cmax mg L-1	tmax h	$t_{1/2}$ hL-1	AUC_{0}-t mgh	AUC_{0-t} $mghL^{-1}$	$AUCRBC^{o-t}$ $AUC\ Plasma_{0-t}$
1	67	1.4	4	8.8	9.68	12.23	0.14
2	62	1.0	4	15.1	16.25	20.62	0.38
3	60	1.9	12	-	15.73	15.73	0.29
4	74	1.7	3	18.5	23.29	28.62	0.90
5	61	1.1	8	17.7	26.10	31.20	0.23
6	64	1.4	1	15.4	15.40	19.74	0.40
7	58	1.4	10	18.7	31.39	39.48	0.43
8	62	1.0	6	19.7	20.68	26.36	0.25
mean	63.5	1.35	6	16.27	19.81	24.24	0.38
SD	5.0	0.32	3.7	3.71	6.94	8.89	0.23

TABLE 5a. PLASMA PHARMACOKINETIC PARAMETERS AFTER A SINGLE ORAL DOSE OF 500MG QUININE BASE WITH 250MG TETRACYCLINE.

Subject	Weight kg	Cmax mg L-1	tmax h	$t_{1/2}$ hL^{-1}	Vz $L\ kg^{-1}$	CLP $Lhkg^{-1}$	AUC_{o-t} mgh L-1	$AUC_{o-\infty}$ $mghL^{-1}$
1	67	2.6	6.0	15.8	2.47	0.10	58.6	67.7
2	62	5.6	2.0	9.1	1.93	0.14	52.5	53.8
3	60	2.2	3.0	25.7	3.72	0.10	59.6	81.9
4	74	1.1	6.0	13.4	5.04	0.26	21.5	25.3
5	61	3.7	6.0	22.3	2.23	0.06	90.5	116.3
6	64	1.9	4.0	9.9	2.85	0.19	31.9	38.5
7	58	2.6	3.0	12.8	2.94	0.16	49.3	53.0
mean	63.7	2.8	4.3	15.5	3.02	0.14	51.9	62.3
SD	5.3	1.4	1.7	6.2	1.00	0.06	22.0	30.0

TABLE 5b. PLASMA PHARMACOKINETIC PARAMETERS AFTER A SINGLE ORAL DOSE OF 500MG QUININE BASE ALONE.

Subject	Weight kg	Cmax mg L-1	tmax h	$t_{1/2}$ hL^{-1}	Vz $L\ kg^{-1}$	CLP $Lhkg^{-1}$	AUC_{0-t} mgh L-1	$AUC_{0-\infty}$ $mghL^{-1}$
1	67	2.8	2.0	7.3	1.90	0.18	39.3	40.3
2	62	3.0	2.0	10.2	2.39	0.16	47.4	48.9
3	60	1.7	4.0	16.6	4.29	0.18	41.0	45.8
4	74	3.0	4.0	7.6	2.33	0.21	30.2	31.3
5	61	4.2	4.0	24.1	1.78	0.05	122.2	56.9
6	64	2.3	4.0	9.3	3.71	0.27	25.5	26.7
7	58	2.9	4.0	12.5	2.61	0.14	54.9	58.6
mean	63.7	2.8	3.4	12.5	271	0.17	51.5	58.3
SD	5.3	0.7	0.9	6.0	0.93	0.06	52.6	44.7